Disposition of S^{35}-Prochlorperazine in the Rat

By BARRIE M. PHILLIPS* and TOM S. MIYA

Prochloroperazine was shown to be distributed in the organs of the rat much like other weak organic bases. Drug absorption was retarded in repeatedly treated rats and certain tissues appeared to play a storage role. Excretion studies revealed that the fecal route plays the major role in prochlorperazine excretion, less than 25 per cent of the administered dose being excreted in the urine of intraperitoneally dosed control rats. A trend toward decreased urinary and increased fecal excretion was observed in repeatedly treated intraperitoneally dosed and orally dosed rats. Excretion *via* the bile was shown to be a major route in intraperitoneally dosed rats. Unchanged prochlorperazine and three metabolites were demonstrated in the urine.

IN RECENT YEARS numerous reports have appeared concerning the metabolic fate of a variety of *N*-substituted phenothiazines. However, with the exception of the recent reports of Symchowicz *et al.* (perphenazine) (1, 2) and Flanagan *et al.* (prochlorperazine) (3), no investigations have been reported regarding the disposition in animals of the piperazine-linked analogs of this class of compounds. The present report deals with studies of the distribution and excretion of prochlorperazine. Since the development of tolerance to this compound following prolonged administration has been reported (4), a portion of the study was designed to demonstrate any changes in drug disposition which might occur following repeated administration. We have reported previously (5) on the excretion of this agent in the experimentally stressed and jaundiced rat.

METHODS AND MATERIALS

Male albino Holtzman rats of from 250 to 350 Gm. body weight were employed in these studies. All animals were allowed food and water *ad libitum* up to the time of drug administration. Repeatedly treated rats received single intraperitoneal daily doses of 25 mg./Kg. of unlabeled prochlorperazine base (as the dimaleate) for 13 days. They were utilized on the fourteenth day. No attempt was made to determine if a pretreatment regimen of this nature would result in the development of tolerance.

S^{35}-Labeled prochlorperazine dimaleate was used in these studies. It was administered orally or intraperitoneally as a 1.25% suspension in 4% acacia in a dose of 25 mg./Kg. (of the base), as was the unlabeled drug. Each rat received 10 μc. of S^{35} per 275 Gm. of body weight. Within the individual studies, all animals were dosed from the same freshly prepared suspension of drug.

Distribution, metabolism, and excretion studies were conducted on orally dosed, acutely intraperitioneally dosed, and repeatedly intraperitoneally dosed rats. Aside from the method of administration and the tissues sampled, the same techniques were employed in each study.

Drug distribution was studied in three groups of 21 animals, each divided into seven groups containing three rats each. In each group the animals were sacrificed at intervals of 1, 2, 4, 8, 16, 24, and 48 hours after drug administration. Samples of blood, brain [brain samples from intraperitoneally dosed rats were divided into the four areas described by Emmerson *et al.* (6), only cerebral tissue was assayed from orally dosed animals], lung, liver, kidney, spleen, fat, heart, skin, and muscle were obtained from each rat. Tissues were prepared for liquid scintillation counting by adding 3 ml. of a 3:1 mixture of approximately 1 *M* hyamine hydroxide and 30% aqueous potassium hydroxide to approximately 100-mg. portions of tissues contained in Wheaton vials. The vials were then heated at 60° for 2 hours. After cooling at room temperature for 12 hours, 1 ml. of glacial acetic acid and 14 ml. of a scintillator solution (XDC) containing naphthalene, 80 Gm./L; 2,5-diphenyloxazole, 10 Gm./L, and 1,4-di [2-(5-phenyloxazolyl)] benzene, 0.5 Gm./L; in a 1:3:3 mixture of xylene, dioxane, and ethylene glycol monoethyl ether were added.

The urinary and fecal excretion of S^{35} was investigated in three groups containing six rats each. After administration of the labeled drug, the rats were placed in stainless steel metabolism cages. Urine excreted by these rats was collected under toluene at 4, 8, 12, 24, 36, 48, 60, 72, 84, and 96 hours; feces were collected at 24, 48, 72, and 96 hours after dosing. Biliary excretion of S^{35} was studied in five animals by inserting a polyethylene cannula into the bile duct under ether anesthesis. When the animal began to recover from the anesthesia, the labeled drug suspension was administered intraperitoneally, and the animals were restrained in Bollman cages (7). Bile excreted by these animals was collected for 24 hours. Urine and bile samples were prepared for liquid scintillation counting as described previously (8). Feces samples were prepared by adding 3 ml. of the hyamine hydroxide-potassium hydroxide mixture to 50-mg. portions of finely ground feces powder in Wheaton vials. The vials were then heated at 60° for 5 hours. After cooling to room temperature, 4 drops of 30% hydrogen peroxide were added to each vial. When the evolution of gas was complete, the vials were heated at 60° for 2 additional hours. After standing at

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room temperature for 12 hours, 2 ml. of glacial acetic acid and 14 ml. of XDC scintillator solution were added to each vial.

The samples prepared as above were cooled to 0° and counted in a liquid scintillation spectrometer for a period sufficient to give less than 2% counting error. Count rates obtained from these samples were corrected to absolute efficiency by a technique developed by Bruno and Christian (9) or by internal standard techniques. A C^{14} internal standard was employed to avoid the problems associated with the relatively short half-life of S^{35}. Appropriate decay corrections were made in the count rates obtained with all S^{35} samples.

Recovery of metabolites from urine and bile was accomplished by extraction of the total volume excreted by the individual rats during the 96- or 24-hour intervals with ethylene dichloride as described by Fishman and Goldenberg (10) for chloropromazine. Hydrolysis of the urine (5–25 ml.) or bile (1–10 ml.) was accomplished by adding equal amounts of 1 *M* pH 5 acetate buffer and B-glucuronidase, 2,000 units per original 5 ml. of urine or 5,000 units per original 5 ml. of bile. The mixtures were then transferred to 50-ml. conical flasks and incubated at 37° for 18 hours in a Dubnoff shaker.

Urine and bile extracts, reconstituted in 1 ml. of ethylene dichloride, were chromatographed on Whatman No. 3 chromatographic paper using the upper phase of an iso-amyl:tertiary-amyl:formic acid:water (5:5:4:8) system. Chromatograms were developed in the absence of light in a nitrogen atmosphere. Prochlorperazine and its metabolites were visualized on these chromatograms by observation under short-wave ultraviolet light and after spraying with the iodoplatinate reagent described by Smith (11).

Corrected count rates obtained in the distribution studies were treated statistically by the method of least squares so that the concentration of radioactivity in tissues with time could be expressed as a straight line. The biological half-life of S^{35} in the various tissues was calculated using first-order rate equations (12). The significance of differences between the levels of radioactivity in the several tissues in a single group of animals and between the rates of disappearance of S^{35} from those tissues was tested by the Newman-Keuls test (13); the error terms employed in these tests were determined by analysis of variance. All other differences were tested by the *t* test.

RESULTS

Distribution.—The data in Table I describe the distribution plots in the various tissues of the three groups of animals. Data for blood are not included, since in all three studies blood levels were extremely low and nonlinear.

In general, the various tissues can be divided into two categories: (*a*) those from which S^{35} leaves relatively rapidly (brain and fat) and (*b*) those from which it disappears more slowly (reticuloendothelial tissues). Kidney, heart, and skin fall intermediate between these two categories (see Table I). However, while there are roughly two rates at which S^{35} is lost from tissues, the average concentrations reached in the tissues vary widely. In orally dosed rats there is no clear-cut delineation between the rates of disappearance of S^{35} from the various tissue; but there are only minor differences resulting in a spectrum of slopes, only the extremes of which differ significantly. S^{35} disappears most rapidly from cerebrum, fat, and muscle, and more slowly from the reticuloendothelial tissues. This was because, at least in part, the levels were linear over fewer time intervals; therefore, a more conservative error term was employed. In the repeatedly treated rats, evels of S^{35} in the cerebrum are significantly greater than levels in the other brain areas examined, whereas in control rats, S^{35} levels reached in cerebrum, midbrain, and hindbrain are not significantly different.

Tests of differences between distribution plots in corresponding tissues indicated that S^{35} disappeared from liver, kidney, heart, and muscle at a significantly more rapid rate in control than in repeatedly treated rats, as illustrated by the longer half-lives in these tissues in the latter animals. In addition, in nearly every tissue of repeatedly treated rats, peak levels of S^{35} are not reached until 2 hours after drug administration, while in control animals peak levels are attained in 1 hour in almost every tissue. The result of this change in the pattern of drug absorption in the major brain areas

TABLE I.—DISTRIBUTION AND S^{35}-BIOLOGICAL HALF-LIVES

Tissue	Av. S^{35} Concn.[a]	Half-Life, Hr.
	Control-i.p.	
Cerebrum	4.21	7.08
Midbrain	3.48	7.82
Cerebellum	3.02	7.79
Hindbrain	3.49	7.79
Lung	41.05	10.75
Liver	48.55	12.06
Kidney	18.19	9.81
Spleen	23.32	11.62
Fat[b]	103.03	6.08
Heart	4.59	9.64
Skin[c]	5.95	9.69
Muscle	2.54	8.81
	Repeated-i.p.	
Cerebrum	7.55	8.66
Midbrain	5.36	8.14
Cerebellum	4.38	8.49
Hindbrain	4.69	7.79
Lung	100.46	12.15
Liver	76.54	18.83[d]
Kidney	31.76	15.10[d]
Spleen	43.65	14.66
Fat[b]	73.10	8.22
Heart	12.83	15.29[d]
Skin[c]	5.75	12.15
Muscle	3.66	10.96[d]
	Oral	
Cerebrum[c]	2.19	8.82
Lung[c]	28.02	11.03
Liver[b]	48.72	26.08
Kidney[b]	15.44	18.63
Spleen[b]	17.25	16.63
Fat[b]	5.87	12.15
Heart[b]	4.49	14.43
Skin[c]	1.98	13.64
Muscle[c]	1.48	12.70

[a] Mean concentrations over all time intervals expressed as micrograms of prochlorperazine base per gram of wet tissue. [b] Linear from 2 to 48 hours. [c] Linear from 4 to 48 hours. [d] Significantly longer (0.05) than in the corresponding tissue in control-intraperitoneal animals.

TABLE II.—MEAN CUMULATIVE PER CENT OF ORIGINAL DOSE OF S^{35} EXCRETED IN URINE AND FECES FOLLOWING ADMINISTRATION OF S^{35}-PROCHLORPERAZINE TO RATS

Route of Excretion	I. p. Control	I. p. Repeated[a]	Oral
		Urine	
4[b]	0.94	1.68	0.92
8	6.98	4.91	3.27
12	9.09	7.09	6.56
24	17.87	12.70	13.35
36	21.49	15.01	14.51
48	22.64	16.24	15.10
60	22.85	16.71	15.54
72	22.98	17.12	15.86
84	23.04	17.33	16.03
96	23.20^{I}	17.62^{II}	16.15
	$\pm 0.61^{c}$	$\pm 0.92^{c}$	$\pm 1.21^{c}$
		Feces	
24	2.75	8.64	42.87
48	56.39	66.35	71.56
72	68.10	76.42	74.27
96	71.51^{III}	78.73^{IV}	75.12
	$\pm 3.45^{c}$	$\pm 2.67^{c}$	$\pm 4.54^{c}$
Total	94.71^{V}	96.34^{VI}	91.27
	$\pm 3.59^{c}$	$\pm 2.73^{c}$	$\pm 5.61^{c}$

[a] Mean of five rats, others the mean of six rats. [b] Hours after drug administration. [c] ± Standard error; I *vs.* II, significant at the 0.01 level; III *vs.* IV and V *vs.* VI, not significant at the 0.05 level.

is that the initial peak levels obtained in control animals are never reached in the repeatedly treated rat, although levels at later time intervals are somewhat higher in repeatedly treated animals. Biological half-lives of S^{35} in tissues of orally dosed rats are 10 to 100% longer than in intraperitoneally dosed control rats, probably the result of a more prolonged drug-absorption time.

S^{35} Excretion.—Table II summarizes the results of the urinary and fecal excretion studies. These data emphasize the importance of the fecal excretion route for prochlorperazine; urinary excretion accounts for less than 25% of the total amount excreted by control rats. A significantly smaller portion of the original dose was excreted in the urine by repeatedly treated rats than by control rats; this was compensated by increased fecal excretion. In the biliary excretion study 36.79 ± 4.72% of the administered dose of S^{35} appeared in the bile in 24 hours.

Metabolite.—Four basic compounds were detected in urine extracts by paper chromatography (R_f values: I = 0.15, II = 0.20, III = 0.38, IV = 0.84). None of metabolite I appeared in the bile. Comparison of the chromatographic behavior of IV with authentic prochlorperazine in the solvent system described indicated it to be unchanged prochlorperazine. No attempt was made to identify the other metabolites. Because of the relatively small percentage of the original dose excreted in the urine, none of these represented a substantial portion of the dose administered. About 65% of the S^{35} excreted in the urine was extractable by the method employed, only an additional 7% was extractable following the treatment with β-glucuronidase. Thus, among the three groups of rats, only 10–16% of the dose of S^{35} was excreted in the urine as organic extractable material. Very little of the S^{35} present is extractable from bile with ethylene dichloride (10%), and incubation with β-glucuronidase does not result in subsequent extraction of an appreciably greater quantity (16%). Metabolites in the various tissue samples could not be determined.

DISCUSSION

The finding that blood levels were low at all time intervals is typical of many phenothiazines (1, 14, 15). One exception is that the brain levels determined in this study were much lower with respect to other tissues than were brain levels of chlorpromazine determined by Salzman and Brodie (14). Symchowicz *et al.* (1) found that the piperazine-linked phenothiazine, perphenazine, was less concentrated in the brain than in reticuloendothelial tissues, but more concentrated than in skeletal muscle. Thus, somewhat lower brain levels may be characteristic of piperazine-linked *N*-substituted phenothiazines.

These findings demonstrate that repeated administration of prochlorperazine does not result in an increased rate of disappearance of drug from the brain, although peak levels are reduced. The fact that S^{35} disappears more slowly from liver, kidney, heart, and muscle in repeatedly treated rats compared with controls suggests that these tissues may play a storage role in the repeatedly treated animal. In addition, there appears to be a decrease in the rate of absorption of the test dose in repeatedly treated rats.

It was anticipated that fecal excretion of prochlorperazine would be an important route since this is typical of organic bases in general. However, it was not expected that there would be such a marked difference in the relative importance of the two routes since Emmerson and Miya (16) found that fecal and urinary routes of excretion of chlorpromazine in the rat were of essentially equal importance. These findings are compatible with those of Flanagan *et al.* (3), although a direct comparison is not possible because of the difference in doses employed. In view of the findings of Symchowicz *et al.* (1), this pattern of excretion should be considered typical of the piperazine-linked phenothiazines. However, while the rate of urinary excretion of prochlorperazine and perphenazine during the initial 24 hours after drug administration is virtually identical, fecal excretion of prochlorperazine occurs at a much slower rate than is the case with perphenazine. Results of the excretion study in orally dosed rats are in agreement with the findings of the distribution study. The decreased urinary excretion of S^{35} and the rapid appearance of S^{35} in large quantities in the feces implies slower absorption of prochlorperazine. This suggestion is supported by the lower tissue levels of S^{35} obtained in orally dosed rats.

The excretion studies revealed a major alteration in drug disposition resulting from repeated dosing with prochlorperazine. The results are similar to the findings of Mellett and Woods (17), who observed a trend toward decreased urinary and increased fecal excretion of morphine in morphine-tolerant rats. In contrast to these findings, however, Emmerson and Miya (16) found that rats repeatedly treated with chlorpromazine excreted more of a test dose of chlorpromazine in the urine and less in the feces than control rats.

Results of the biliary excretion study imply that the disposition of material excreted into the duodenum *via* the bile does not involve simple direct excretion in the feces. Excretion of large amounts of S^{35} into the duodenum *via* the bile does not lead to fecal excretion of an appreciable quantity of S^{35} during the initial 24 hours after intraperitoneal drug administration. However, about five times as much is excreted in the feces during the first 24 hours when the drug is introduced directly into the gastrointestinal tract (oral dosing). The finding that none of the administered dose is excreted in the bile as metabolite I implies that this metabolite represents further alteration of either metabolite II or III.

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Thin-Layer Radiochromatographic Study of Prochlorperazine Photodeterioration

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Procedures to determine microquantities of photochemical deterioration products of prochlorperazine were developed by using thin-layer chromatography in combination with the radioisotope tracer technique. Up to 11 products were separated on a silica gel thin-layer plate using ethylene dichloride-methanol-ammonia as the solvent. The separated radioactive spots were scraped off the plate, suspended in a Thixcin scintillator gel, and counted. Quantitative results for each product indicated that complex reactions are involved in the photochemical deterioration of prochlorperazine.

PHENOTHIAZINE DERIVATIVES are decomposed by light into various oxidative and cleavage compounds (1–3). They are metabolized by *N*-methyl and side chain cleavage and formation of sulfoxides, phenols, free radicals, and *N*-oxides (4). Several analytical procedures have been used for separating and identifying phenothiazine derivatives and metabolites, especially those of chlorpromazine (4). The most important of these was paper chromatography in combination with spectrophotometry and the radioisotope tracer technique.

Thin-layer chromatography recently has become widely recognized because of many advantages over paper chromatography, such as speed, simplicity, and high sensitivity. The combination of thin-layer chromatography and the radioisotope tracer technique is especially well adapted to very small samples. This combination was reported by Snyder and Stephens (5) in which C^{14} and H^3 labeled lipids were analyzed. The purpose of this study was to use thin-layer chromatography combined with the radioisotope tracer technique for the microquantitative study of the photochemical deterioration of prochlorperazine.

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EXPERIMENTAL

Materials.—Fixed thickness chromatofilm assembly, ascending (model 200-1, Research Specialties Co., Richmond, Calif.) with 8 × 8-in. glass plates was used. Also used were silica gel G (Merck, Germany); prochlorperazine ethanedisulfonate and prochlorperazine-S-35 ethanedisulfonate (Smith Kline and French Laboratories, Philadelphia, Pa.) (the purity of the labeled compound was 99.1%); a Beckman model DB spectrophotometer; a Packard model 314-X Tri-Carb liquid scintillation spectrometer; and solvents and chemicals of reagent grade or equivalent.

Thin-Layer Chromatography.—Aqueous solutions of unlabeled prochlorperazine ethanedisulfonate (0.010, 0.10, and 5.0 mg./ml.) were irradiated by sunlight for 2 hours. The solutions became colored shortly after exposure and darkened further upon continued exposure. The change in the absorption